DIFFERENTIAL EFFECTS OF DCMU ON THE LIGHT-INDUCED ABSORBANCE CHANGES AT 518 nm, H^+ UPTAKE AND CYCLIC PHOTOPHOSPHORYLATION IN ISOLATED CHLOROPLASTS

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1. Introduction

The light-induced absorbance change at 518 nm in isolated chloroplasts was interpreted by Witt et al. [1] as the expression of an electric potential across the chloroplast membrane. Hauska et al. [2] showed that under conditions of complete inhibition of the pH rise, both ATP formation and the 518 shift are retained. However, correlation between the 518 shift and ATP formation was criticized by Neumann et al. [3]. The purpose of this communication is to describe conditions under which cyclic ATP formation is almost completely inhibited while the extent of the light induced absorbance change at 518 nm and H^+ uptake have not been altered.

2. Methods

Chloroplasts and digitonin-treated subchloroplast particles (SCP) were prepared from spinach leaves as previously described [4]. Tris-treated chloroplasts were prepared according to Yamashita and Butler [5]. Light-induced absorbance changes at 518 nm were measured in an Aminco–Chance dual wavelength spectrophotometer modified for side-illumination. The reference wavelength was 540 nm. A red filter (Corning #2403) and two heat filters were placed in the illumination light path and a blue filter (Corning #9782) was placed in front of the phototube. Light intensity at the level of the cuvette was about 2×10^5 ergs/cm^2/sec. The reaction mixture contained the following in μmoles; Tricine (pH 8), 50; NaCl, 50; $MgCl_2$, 20; NaP_i, 10; ADP, 3; PMS, 0.09; and approx. 10^6 cpm of ^{32}P in a final volume of 3.0 ml. After 30 sec of illumination, aliquots were removed and ^{32}P incorporation was assayed according to Avron [6]. Light induced proton uptake was measured according to the procedure of Neumann and Jagendorf [7].

3. Results

Fig. 1 shows the effect of increasing concentrations of DCMU on absorbance changes at 518 nm and photophosphorylation in isolated chloroplasts. The figure shows clearly that while ATP formation is strongly inhibited by DCMU, little effect is seen on the extent of the light-induced absorbance change at 518 nm. It may be emphasized that both effects were measured in the same cuvette.

Table 1 demonstates the effect of ascorbate and DCMU on photophosphorylation and the absorbance change at 518 nm in Tris-treated chloroplasts and digitonin-treated SCP. In Tris-treated chloroplasts, ascorbate accelerated ATP formation more than 300 times, while the extent of the 518 nm absorbance change was only slightly increased. Digitonin-treated SCP were similar to chloroplasts in that they were not influenced by addition of ascorbate and only ATP formation was inhibited by DCMU.

Fig. 2 illustrates the effect of DCMU on proton uptake induced by red light in the presence of PMS. In

Abbreviations. DCMU: 3-(3,5-dichlorophenyl)-1,1-dimethylurea; PMS: phenazine methosulfate.

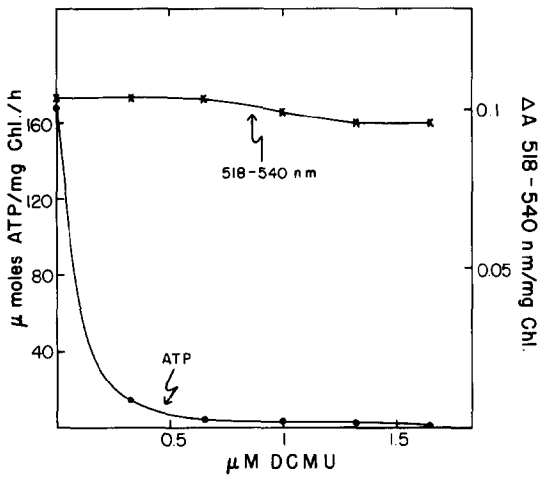

Fig. 1. Influence of DCMU on photophosphorylation with PMS and on the extent of light induced absorbance changes at 518 nm. The experiment was performed as described in Methods, with a chlorophyll content of 68 μg.

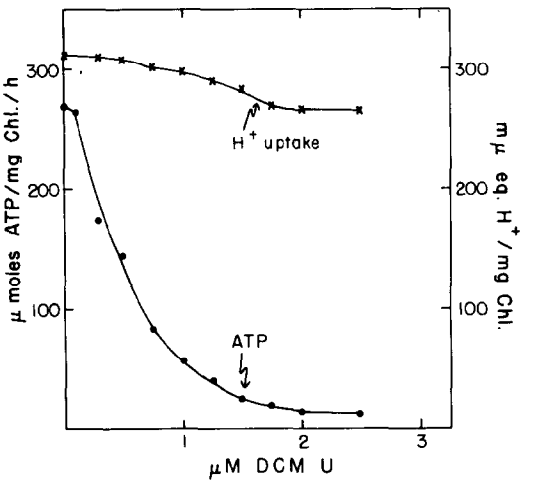

Fig. 2. Effect of DCMU on ATP formation and proton uptake in red light. The reaction mixture for H^+ uptake contained, in a final volume of 2 ml, 100 μmoles of NaCl and 0.03 μmoles of PMS. It also contained a chloroplast suspension equivalent to 88 μg chlorophyll. The initial pH was adjusted to pH 7. Actinic light was passed through a water bath heat filter and red filter (Corning #2403) providing a light intensity of 2×10^5 erg per cm^2 per sec at the level of the reaction mixture. The temperature was kept at 25° by passing water through a jacket.

The formation of ATP was assayed under the same conditions. The chloroplasts were illuminated for 30 sec. The reaction mixture contained in a total volume of 2 ml: 33 μmoles Tricine, 33 μmoles NaCl, 13 μmoles $MgCl_2$, 6.7 μmoles P_i, 2 μmoles ADP, 0.03 μmoles PMS and approx. 2×10^6 cpm of ^{32}P. It also contained a chloroplast suspension equivalent to 88 μg of chlorophyll. The initial pH was adjusted to pH 7.

Table 1
Photophosphorylation and 518 nm absorbance changes in Tris- and digitonin-treated chloroplasts.

	ATP (μmoles/mg chl/hr)	ΔA 518–540 /mg chl
Chloroplasts	298	0.132
Chloroplasts + 1 mM ascorbate	320	0.132
Tris treated chloroplasts	0.4	0.066
Tris treated chloroplasts + 1 mM ascorbate	136	0.074
Digitonin treated SCP	207	0.126
Digitonin treated SCP + 1 mM ascorbate	190	0.126
Digitonin treated SCP + 1 μM DCMU	37	0.126

Experimental conditions as described in Methods.

this experiment, H^+ uptake and ATP formation were measured at pH 7, but with different reaction mixtures. While ATP formation was strongly inhibited by DCMU, proton uptake was hardly affected. Proton uptake, measured at pH 6, was also resistant to DCMU. Proton uptake, measured in the presence of ADP, P_i and $MgCl_2$ according to Karlish and Avron [8], was also much more resistant to DCMU than was ATP formation.

4. Discussion

The light-induced absorbance change at 518 nm in chloroplasts and chromatophores was interpreted as a

reflection of their membrane potential.

Fig. 1 shows that DCMU at μM concentrations completely blocks PMS-mediated ATP formation in red light while the extent of the 518 nm shift is hardly affected. By adding ascorbate or by brief exposure to white light, ATP formation was completely restored while no further spectral changes at 518 nm could be detected. If one assumes that DCMU in the μM range blocks specifically electron transport from water, PMS-mediated cyclic electron flow would have to be "forced" by reducing part of the PMS [9]. The data presented in fig. 1 suggest that very limited cyclic electron flow, which is not enough for photophosphorylation, was able to produce the full signal at 518 nm. Reducing the PMS, either by addition of a reducing agent, or by illumination with white light leads to rapid cyclic electron flow and coupled ATP formation without changing the extent of the 518 nm signal. The data in table 1 with digitonin-treated SCP and Tris-treated chloroplasts are consistent with this explanation. This suggests that if the extent of absorbance changes at 518 nm in chloroplasts do in fact indicate the existence of a membrane potential, then this potential cannot in itself drive ATP formation.

The explanation for the resistance of H^+ uptake to increasing concentrations of DCMU in red light is more difficult. Since massive proton uptake was observed on illumination with red light in the presence of DCMU, it cannot be explained by residual electron transport. Assuming that DCMU does not act as an energy transfer inhibitor, and assuming also that a proton gradient can lead to efficient ATP formation, there is no simple explanation to this phenomenon. It cannot be claimed that the small inhibition of proton uptake observed in these experiments decreased the proton gradient below the threshold amount which is sufficient for ATP formation. By decreasing the light intensity, illuminated chloroplasts, which had a lower proton uptake than those used in the present study retained appreciable rates of ATP formation in the presence of 2.5 μM DCMU. It is unlikely that this concentration of DCMU will change drastically the buffer capacity of the internal volume of the chloroplasts, and therefore lead to inhibition of ATP formation [10].

The explanations that seem reasonable are the following: i) A pH gradient cannot lead to rates of ATP formation comparable to those measured in steady state photophosphorylation (but see [11]). ii) In order to get high rates of photophosphorylation, one must not only have a sufficient pH gradient, but also an appropriate redox state of the electron carriers (but see [12]). iii) Proton uptake and ATP formation are driven by two different systems. In red light and the presence of DCMU only proton uptake remains, while in the presence of any reductant (white light, ascorbate or photosystem II without DCMU), both reactions proceed.

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References

[1] H.T. Witt, B. Rumberg and W. Junge, Colloq. Ges. Biol. Chem. 19 (1968) 262.
[2] G.A. Hauska, R.E. McCarty and J.S. Olson, FEBS letters 7 (1970) 151.
[3] J. Neumann, B. Ke and R.A. Dilly, Plant Physiol. 46 (1970) 86.
[4] N. Nelson, Z. Drechsler and J. Neumann, J. Biol. Chem. 245 (1970) 143.
[5] T. Yamashita and W.L. Butler, Plant Physiol. 43 (1968) 1978.
[6] M. Avron, Biochim. Biophys. Acta 40 (1960) 257.
[7] J. Neumann and A.T. Jagendorf, Arch. Biochem. Biophys. 107 (1964) 109.
[8] S.J.D. Karlish and M. Avron, Nature 216 (1967) 1107.
[9] G.A. Hauska, R.E. McCarty and E. Racker, Biochim. Acta 179 (170) 206.
[10] N. Nelson, H. Nelson, Y. Naim and J. Neumann, Arch. Biophys. 145 (1971) 263.
[11] Y. Nishizaki and A.T. Jagendorf, Biochim. Biophys. Acta 226 (1971) 172.
[12] C.D. Miles and A.T. Jagendorf, Biochemistry 9 (1970) 429.